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鹭科鸟类性别鉴定方法及白鹭和黄嘴白鹭雏鸟种群性别比例的研究

Sex identification methods in ardeid birds and sex ratio in the nestling populations of Little Egret and Chinese Egret

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中文摘要

本实验在筛选鹭科鸟类性别鉴定分子标记的基础上，利用这种方法检测白鹭和黄嘴白鹭雏鸟种群的性别比例，分析白鹭和黄嘴白鹭的雏鸟性别与产卵顺序之间的关系，并进一步分析鹭科鸟类 W 染色体以及 Z 染色体的 CHD 片段序列并尝试用于亲缘关系及系统进化的研究。

本文以鹳形目 5 种鹭科鸟类和 1 种鹮科鸟类的组织为实验材料，采用 PCR 方法扩增其性别基因相关片段，探讨鹭科鸟类性别的分子鉴定方法。通过测定 3 对已知性别的白鹭（*Egretta garzetta*）、岩鹭（*Egretta sacra*）和黄嘴白鹭（*Egretta eulophotes*）以及 12 只未知性别的夜鹭（*Nycticorax nycticorax*）、池鹭（*Ardeola bacchus*）、白鹭、牛背鹭（*Bubulcus ibis*）、黑脸琵鹭（*Platalea minor*）的性别基因 EE0.6 或 CHD 上的基因相关片段并进行比较。结果表明，通过扩增 EE0.6 或 CHD 基因片段的性别鉴定分子方法都能够适用于鹭科鸟类的性别鉴定，由此能够解决鹭科鸟类雌雄外形同色而难以从外貌上区分其性别的问题；在 EE0.6 和 CHD 两种鹭类性别鉴定方法中，CHD 方法不仅准确而且比 EE0.6 方法更为简单。

本文利用引物 P_2、P_8 通过扩增 CHD 基因特定片段鉴定了三地共 361 只个体的性别，对扩增条带不明显的个体利用引物 2550F、2718R 进行校正。结果表明，白鹭雏鸟种群（鸡屿岛，共 69 只）的性别比例具明显的雌性偏向（雌性比例为 0.6522±0.4798(平均值±标准差)，$P<0.05$）；黄嘴白鹭雏鸟种群（杏仁砣和小菜屿，共 213 只）的性别比例没有明显的性别偏向（雌性比例为 0.4507±0.4987，$P>0.05$），其中，杏仁砣黄嘴白鹭种群雏鸟（154 只）的性别比例没有明显的性别偏向（雌性比例为 0.5130±0.5015，$P>0.05$），而小菜屿黄嘴白鹭雏鸟种群（59 只）的性别比例则有明显的雄性偏向（雌性比例为 0.2881±0.4568(mean ± SD)，$P<0.01$）。按巢统计的性别比例（巢内性别比例）与种群性别比例的结果相似。白鹭（17 巢鸡屿岛）的巢内性别比例具明显的雌性偏向（0.6333±0.2756，$P<0.05$），黄嘴白鹭（杏仁砣+小菜屿共 65 巢）的巢内性别比例无明显的性别偏向（0.4648±0.3108，$P>0.05$）；其中，杏仁砣黄嘴白鹭（45 巢）的巢内性别比例无明显的性别偏向（0.5571±0.2941，$P>0.05$）；但是小菜屿黄嘴白鹭（20 巢）的巢内性别比例具明显的雄性性别偏向（0.2708±0.2485，$P<0.01$）。比较三地种群的雌性比例关系为白鹭（鸡屿）＞黄嘴白鹭（杏仁砣＋小菜屿）($P<0.01$)，

鸡屿岛白鹭＞杏仁砣黄嘴白鹭（P>0.05），鸡屿岛白鹭＞小菜屿黄嘴白鹭（P<0.05），杏仁砣黄嘴白鹭＞小菜屿黄嘴白鹭（P<0.01）；三地巢内雌性比例关系为（鸡屿）＞黄嘴白鹭（杏仁砣＋小菜屿）(P<0.05),鸡屿岛白鹭＞杏仁砣黄嘴白鹭（P〉0.05），鸡屿岛白鹭＞小菜屿黄嘴白鹭（P<0.01），杏仁砣黄嘴白鹭＞小菜屿黄嘴白鹭（P<0.01）。

本文还探讨白鹭和黄嘴白鹭的产卵顺序与雏鸟性别之间的关系。结果发现，白鹭（鸡屿岛）第一至第五枚卵的雌性比例分别为 0.556±0.511（n=17），0.778±0.428（n=17），0.611±0.502（n=17），0.463±0.497（n=13）和 1.000（n=1）。黄嘴白鹭（杏仁砣+小菜屿）第一至第五枚卵的雌性比例分别是 0.403±0.494(n=68),0.508±0.504(n=62),0.482±0.504(n=48),0.539±0.508（n=22），1.000（n=3）。在黄嘴白鹭的两个种群当中，杏仁砣黄嘴白鹭第一至第五枚卵的雌性比例分别为 0.438±0.501（n=45），0.652±0.482（n=42），0.595±0.498（n=34），0.565±0.507（n=20），1.000（n=3）；小菜屿黄嘴白鹭第一到第四枚卵的雌性比例分别是 0.333±0.476(n=23),0.158±0.375(n=20),0.235±0.437（n=14），0.5±0.577（n=2）。可见，巢内窝卵数为 5 枚的白鹭和黄嘴白鹭的最后一枚卵都是雌性。除了白鹭（鸡屿岛）第四枚卵的雌性比例＜杏仁砣+小菜屿黄嘴白鹭（P>0.05）或杏仁砣黄嘴白鹭（P>0.05）或小菜屿黄嘴白鹭（P>0.05）以外，第一、三枚卵的雌性比例都是白鹭＞黄嘴白鹭（杏仁砣+小菜屿）（P>0.05）或杏仁砣黄嘴白鹭（P>0.05）或小菜屿（P<0.05），而杏仁砣黄嘴白鹭第一到第四枚卵的雌性比例都大于小菜屿黄嘴白鹭（P<0.05）。鸡屿岛白鹭和杏仁砣黄嘴白鹭都是第二枚卵的雌性比例最高，而小菜屿黄嘴白鹭第二枚卵的雌性比例最低。

本文最后对黄嘴白鹭、牛背鹭和黑脸琵鹭的 W 染色体上扩增的 CHD 片段，以及黄嘴白鹭、大白鹭、中白鹭、牛背鹭、夜鹭和黑脸琵鹭 Z 染色体上扩增的 CHD 片段进行序列分析并用于亲缘关系及系统进化的研究。发现引物 P_2、P_8 扩增的 CHD-W 基因的片段较为保守，而扩增的 CHD-Z 基因片段变异较大，而且在对黄嘴白鹭的性别鉴定过程中发现 Z 染色体长度多态现象。因此，这些片段不适于作为亲缘关系和系统进化研究的分子标记。

综上所述，本文建立了鹭科鸟类的快速、简单、准确的性别鉴定方法，解决

了鹭科鸟类雏鸟不能利用外部形态进行性别准确辨别的难题。本研究显示，白鹭种群的性别比例和巢内性别比例具明显的雌性偏向（P<0.05 ），白鹭的种群性别比例和巢内性别比例（雌性比例）大于黄嘴白鹭（P<0.05），该结果可能是解释白鹭种群繁殖力和增长速度大于黄嘴白鹭的原因之一。白鹭的种群性别比例和巢内性别比例（雌性比例）大于黄嘴白鹭的原因则是在于白鹭的第一至第三枚卵的雌性比例大于黄嘴白鹭。白鹭的种群性别比例和巢内性别比例（雌性比例）大于黄嘴白鹭，杏仁砣黄嘴白鹭的种群性别比例和巢内性别比例又大于小菜屿黄嘴白鹭（P<0.01），而鸡屿岛白鹭亲鸟的种群数量＞杏仁砣黄嘴白鹭＞小菜屿黄嘴白鹭，提示鹭鸟种群大小与雌性比例之间可能存在着正比关系。上述结果填补了国内外关于鹭科鸟类性别研究中的空白，也为今后进一步开展鹭鸟繁殖和保护奠定了理论和技术的基础。

关键词 鹭科鸟类；性别鉴定；性别比例；产卵顺序

Abstract

In this paper, molecular markers for sex identification in ardeid birds were screened and the sex ratio in the nestling populations of Little Egret and Chinese Egret were measured. The relationships between nestling sex ratio and laying order in the broods of Little Egret and Chinese Egret were analyzed. And the sequences of CHD-W gene and CHD-Z gene in ardeid birds were analyzed and tried to study their relative and phylogenetic evolution.

Molecular methods of sex identification were studied by amplifying sex gene sequences in five species of ardeidae and one species of threskiornithidae in ciconiiformes birds. Sex gene sequences of EE0.6 or CHD were amplified and compared in three pairs of Little Egret (*Egretta garzetta*), Eastern Reef Heron （*Egretta sacra*） and Chinese Egret（*Egretta eulophotes*） whose sexes had been identified by dissection, and in twelve individuals of Night Heron (*Nycticorax nycticorax*), Cattle Egret (*Bubulcus ibis*),Chinese Pond Heron (*Ardeola bacchus*), Little Egret and Black-faced Spoonbill (*Platalea minor*) whose sexes were unidentified. The results showed that molecular methods of sex identification by amplifying sex gene sequence of EE0.6 or CHD are suitable for the sex identification of ardeid birds, and thus can solve the problem that the sexes of ardeid birds are difficult to be distinguished by their appearance as both male and female ardeid birds have the similar feather color. Furthermore, in both EE0.6 and CHD molecular methods of ardeid sex identification, the CHD method is not only more accuracy but also simpler than EE0.6 method.

In this study, 361 ardeid birds in three populations of Little Egret at Jiyu island, Chinese Egret at Xingrentuo and Xiaocaiyu islands were sexed by amplifying CHD gene sequences using P_2 and P_8. When the amplified bands were not clear, the sex identifications were corrected by using 2550F and 2718R. The result showed that the population sex ratio of Little Egret (69 individuals in total at Jiyu island) was biased to female(the female proportion was 0.6522±0. 4798, P<0.05)and that of Chinese Egret (213 individuals in total in both Xingrentuo and Xiaocaiyu) was no biased (the female proportion was 0.4507±0.4987, P>0.05)in which the population sex ratio at Xingrentuo island was no biased (the female proportion was 0. 5130±0. 5015, $P>0.05$) but the population sex ratio at Xiaocaiyu island was biased to male (the female proportion was 0. 2881±0. 4568, $P<0.01$) . The further analysis of brood sex ratio had the results similar to the population sex ratio. The brood sex ratio in the population of Little Egret (Jiyu island, 17 broods) was biased to female（brood sex ratio was 0.6333 ± 0.2756 , $P<0.05$) and that of Chinese Egret (Xingrentuo population and Xiaocaiyu population, 65 broods in total) was no biased (0.4648±0.3108，$P>0.05$) in which the brood sex ratio in the population of Xingrentuo island (45 broods) was no biased (0.5571 ± 0.294, $P>0.05$) but that of Xiaocaiyu island (20 broods) was biased to male (0.2708 ± 0.2485, $P<0.01$).The relationship of the proportion of females among the three populations were Little Egret (Jiyu island)> Chinese Egret (Xingrentuo and Xiaocaiyu islands in total)($P<0.01$), Little Egret on Jiyu island> Chinese Egret on Xingrentuo （$P>0.05$）, Little Egret on Jiyu island> Chinese Egret on Xiaocaiyu（$P<0.01$）, Little Egret on Jiyu island> Chinese Egret on Xiaocaiyu（$P<0.05$）; The relationship of the brood sex ratio (proportion of females) among the three populations were Little Egret (Jiyu island)> Chinese Egret (Xingrentuo and Xiaocaiyu islands in total)($P<0.05$), Little Egret on Jiyu island> Chinese Egret on Xingrentuo （$P>0.05$）, Little Egret on Jiyu island> Chinese Egret on Xiaocaiyu（$P<0.01$）, Little Egret on Jiyu island> Chinese Egret on Xiaocaiyu（$P<0.01$）.

This paper also analyzed the relationship between the sex ratio (females proportion) and the laying order in the broods of Little Egret and Chinese Egret. The result found that the female proportion of Little Egret in the laying order from the first to the fifth was 0.556 ± 0.511（n=17）, 0.778 ± 0.428（n=17）, 0.611 ± 0.502（n=17）, 0.463 ± 0.497（n=13）, and 1.000（n=1） respectively, and the female proportion of Chinese Egret in the laying order from the first to the fifth was 0.403 ± 0.494（n=68）, 0.508 ± 0.504（n=62）, 0.482± 0.504（n=48）, 0.539 ± 0.508（n=22）, 1.000（n=3） respectively. In two populations of Chinese Egret, the female proportion in the laying order from the first to the fifth was 0.438 ± 0.501（n=45）, 0.652 ± 0.482（n=42）, 0.595 ± 0.498（n=34）, 0.565 ± 0.507（n=20）, 1.000（n=3） for Xingrentuo population and

0.333 ± 0.476（n=23）, 0.158 ± 0.375（n=20）, 0.235 ± 0.437（n=14）, 0.5 ± 0.577（n=2） for Xiaocaiyu population. The results showed that the sex of last egg in the brood was female when the clutch size of the brood was 5. The female proportion of Little Egret in the laying order from the first to the third was higher than that of Chinese Egret (Xingrentuo and Xiaocaiyu islands in total, (P>0.05) or that of Chinese Egret at Xingrentuo island (P >0.05) or that of Chinese Egret at Xiaocaiyu island (P <0.05) except that the female proportion of Little Egret in the fourth laying order was lower than that of Chinese Egret (Xingrentuo and Xiaocaiyu islands in total, (P>0.05) or that of Chinese Egret at Xingrentuo island (P>0.05) or that of Chinese Egret at Xiaocaiyu island (P<0.05) . The female proportion of Chinese Egret at Xingrentuo island in the laying order from the first to the fourth was higher than that of Chinese Egret at Xiaocaiyu island (P <0.05).The female proportion of the second laid egg was the highest in Little Egret at Jiyu island or in Chinese Egret at Xingrentuo island but was the lowest in Chinese Egret at Xiaocaiyu island.

At last in this paper, the sequences of CHD-W gene in Chinese Egret, Cattle Egret and Black-faced Spoonbill，and the sequences of CHD-Z gene in Chinese Egret, Large Egret (*Egretta alba*), Intermediate Egret (*Egretta intermediate*), Cattle Egret, Night Heron and Black-faced Spoodbill were analyzed and used to study their relative and phylogenetic evolution . It was found that the sequences amplified from the CHD-W gene using primers P_2、P_8 were more conservative, the sequences amplified from the CHD-Z gene had many variation, and that the length of Z chromosome had polymorphyism during the sex identification of Chinese Egret. The results indicated that these sequences were not the suitable molecule markers for their relative and phylogenetic study.

In summary, the quick, simple and accurate molecular methods for sex identification of ardeid birds were established in this paper to solve the problem that the ardeid birds are difficult to be sexed by their appearance as both their males and females has the similar feather color. The population sex ratio and brood sex ratio (female proportion) of Little Egret was biased to female（P<0.05）and higher than those of Chinese Egret ($P<0.05$) might explain why the population reproductivity and increase rate in Little Egret was higher than that in Chinese Egret. That the population sex ratio and brood sex ratio (female proportion) of Little Egret was higher than those of Chinese Egret might result from that the female proportion of Little Egret in the laying order from the first to the third was higher than that of Chinese Egret. It is suggested that the population size had the positive effect on the female proportion as the population sex ratio and brood sex ratio (female proportion) of Little Egret was higher than those of Chinese Egret, the population sex ratio and brood sex ratio in the population of Xingrentuo island was higher than that of Xiaocaiyu island ($P<0.05$), the parent population size of Little Egret at Jiyu island was higher than that of Chinese Egret at Xingrentuo island, and the parent population size of Chinese Egret at Xingrentuo island was higher than that of Chinese Egret at Xiaocaiyu island. The results above supplied a gap in the study of sex identification and sex ratio in ardeid birds and supported the academical and technical groundwork for the further researches on the reproductivity and protection of the ardeid birds.

Key words ardeid birds; sex identification; sex ratio; laying order